



# Decontamination of Biological Agents from the Surface of Materials of Military Importance

*A. Schilling, B.W. Gutting, A.F. Slaterbeck, W. Vo, R.S. Mackie, A. Sobota, L. Sobota, S.M. Kuhstoss, K.S. Gaske, and A.N. Rayms-Keller*

*Naval Surface Warfare Center Dahlgren Division  
Dahlgren, Virginia*



Report Documentation Page				Form Approved OMB No. 0704-0188	
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE <b>19 NOV 2003</b>		2. REPORT TYPE <b>N/A</b>		3. DATES COVERED <b>-</b>	
4. TITLE AND SUBTITLE <b>Decontamination of Biological Agents from the Surface Materials of Military Importance</b>				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) <b>Naval Surface Warfare Center Dahlgren Division Dahlgren, Virginia</b>				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT <b>Approved for public release, distribution unlimited</b>					
13. SUPPLEMENTARY NOTES <b>See also ADM001851, Proceedings of the 2003 Joint Service Scientific Conference on Chemical &amp; Biological Defense Research, 17-20 November 2003. , The original document contains color images.</b>					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT <b>UU</b>	18. NUMBER OF PAGES <b>19</b>	19a. NAME OF RESPONSIBLE PERSON
a. REPORT <b>unclassified</b>	b. ABSTRACT <b>unclassified</b>	c. THIS PAGE <b>unclassified</b>			



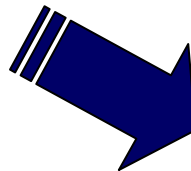
# *Outline*

- Objectives
- Requirements & Guidelines
- Test Challenges
- Procedures
- Results & Conclusions
- Future Issues



# *Objectives*

- Develop method to determine the efficacy of candidate decontaminants on surfaces of military importance
- Begin to bridge the gap between laboratory testing and ‘real world’ application

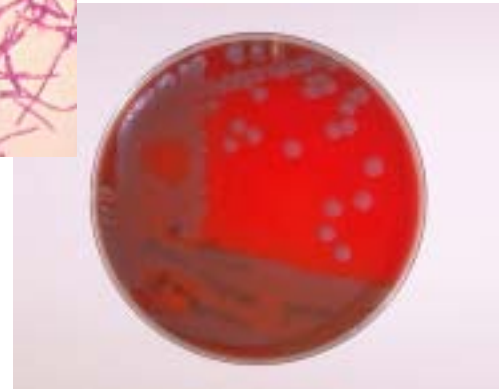




# *Requirements & Guidelines*



- Utilize decontamination procedures in FM 3-5 as a guideline, modifying for the evaluation of decontaminants
- Provide quantitative assessment of decontaminant(s) efficacy





# *Requirements & Guidelines*



## **Test Layout**

- Surfaces
  - Sand, soil, concrete, asphalt
    - ~1 cm<sup>2</sup> surface area
    - Approximately 1 inch (2.54 cm) deep
- Challenge levels
  - *Bacillus anthracis* spores: 10<sup>6</sup> spores/cm<sup>2</sup>  
(JSFDS TEMP: Table 1-6)
- Replicates: Five
- Decontaminant amounts (FM 3-5)
  - Based on amount of decontaminant required for 10 g/m<sup>2</sup> chemical agent challenge (50:1 w/w)



# *Requirements & Guidelines*



## **Test Layout (cont.)**

- Allow contamination to remain 60 min prior to decontamination (NATO requirements AEP 7)
- Decontaminant contact time: 30 min. (FM 3-5)



# *Test Challenges*

- Agent application to material surface
- Decontaminant application to the surface
- Agent recoverability from the surface
- Distinguish between bacteriostatic and bacteriocidal effect
- Reduction of background contamination on surfaces
- Effective quench/neutralization of decontaminant after 30 minutes





## *Procedure*

- Select appropriate method to quench decontaminant
  - Example: For oxidizers, consider a reducing agent, such as sodium metabisulfite or sodium thiosulfate
  - Determine appropriate concentration of neutralization solution
    - Use an excess of neutralizer based upon molar ratios
    - Assay solution to verify absence of active component after neutralization
- Neutralize decontaminant and test on agent
  - Demonstrate minimal effect of neutralized decontaminant on viability of biological agent
  - Demonstrate recoverability of spores from surfaces

# *Procedure*

## **Eliminate background contaminants from the surfaces**

Spiked and untreated controls were tested



### *Soil & Sand*

- Autoclaved 60 minutes at 132°C , 28.5 psi, on a dry cycle
- Dried for 2 hours at 120 °C in dry convection oven

### *Asphalt & Concrete*

- Boiled for 5 minutes
- Dried at 120 °C in dry convection oven
  - Asphalt – 2 hours
  - Concrete – 1 hour



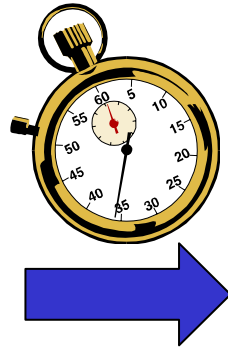
# *Procedure*

**Add Agent**

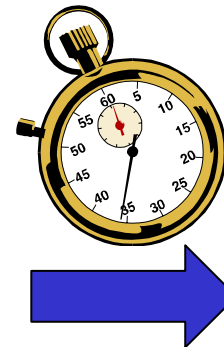
**Add Decontaminant**

**Neutralize**

**60 minutes**



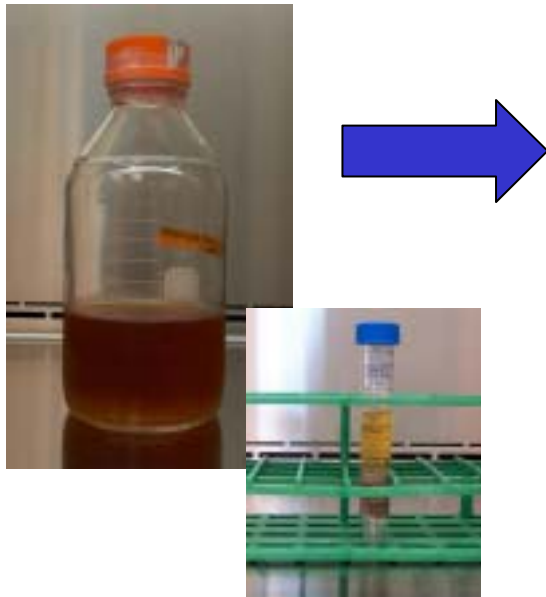
**30 minutes**



# *Procedure*

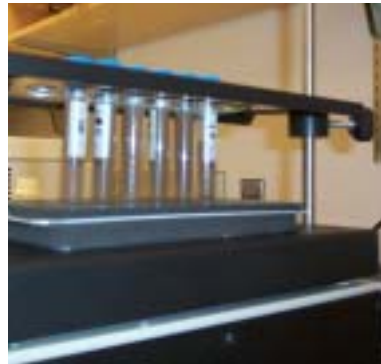
## Add Media

- Final volume = 5 ml



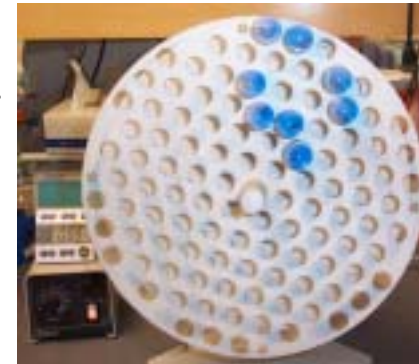
## Vortex

- Sand & soil



## Rotating Shaker

- Concrete & asphalt



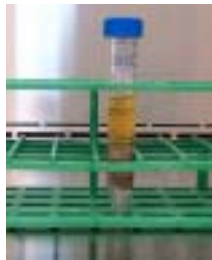
or

Viability Assay



## *Procedure*

- Serial dilutions from each sample
  - Perform dilutions in media (900  $\mu$ l media per tube)
- Plate 100  $\mu$ l from each dilution tube, in duplicate
- Incubate at 37°C
  - Plates – 48 hours
  - Dilution tubes – up to 20 days (determined by test deadline)
  - Sample containing material - incubate with shaking

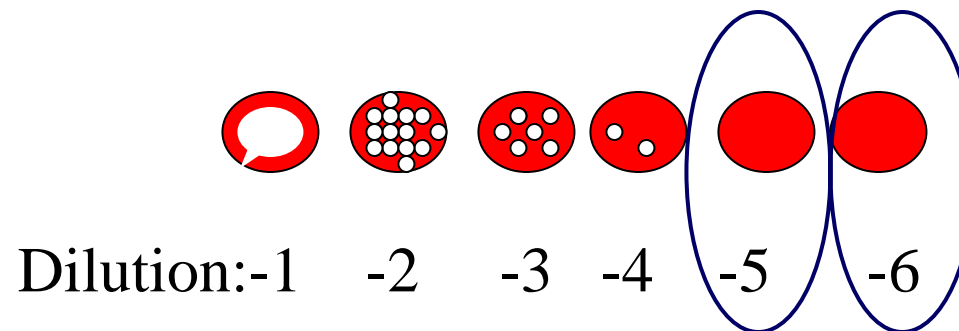




# *Procedure*

## **Determine Efficacy of Decontaminant**

- Count colony forming units
- Select highest two dilutions where zero growth is observed
  - Pipette remaining volume in each corresponding dilution tube into fresh 25 ml of media
  - Allow tubes to incubate in shaker/incubator for 48 hrs
  - Subsample tubes and plate in duplicate on appropriate media
  - Incubate plates and count colony forming units





# *Results & Conclusions*

- To date, we have tested this procedure on:
  - Peroxygen-based decontaminants
    - Alkaline
    - Acidic
  - Hypochlorite-based decontaminants
- Recoverability of biological agents from surfaces treated with *neutralized* decontaminant
  - Not significantly different from untreated surfaces ( $p < 0.05$ )
  - Minimal variability within replicates ( $< 0.5$  log)
- Decontaminant efficacy results were consistent (minimal variability) for plastic and stainless steel substrates
- Variable decontaminating results were observed ( $> 2$  log difference) when asphalt, concrete, soil or sand surfaces were treated with *active* decontaminant





## *Future Issues*

- Porous surfaces (asphalt, concrete, soil, sand, etc.)
  - Interaction between biological agent and decontaminant
  - Interaction between decontaminant and surface materials
  - Aggregation of spores
- Application of agent and decontaminant
- Number of days to incubate dilution tubes
- Media selection
- Standardization of procedures
- Validation







# *Acknowledgements*



We thank the Joint Service Family of Decontamination Systems (JSFDS) Program for their support.

***Backup Slides***



# *References*

## **NATO References**

Quadripartite Standardization Agreements (QSTAG),  
Standard 747, Edition 2, AEP-7. *NBC Survivability  
Acceptance Criteria, Design Guidelines, and Test  
Procedures for Defense Equipment Decontamination  
Survivability Criteria for Military Equipment, Section II.  
Acceptance Criteria.*

## **FM 3-5 References**

### **Decontamination Stations**

“Detailed Equipment Decon” section

- Pages 4-18 through 4-22
- Pages 4-19 through 4-23 (Change 1, 31 Jan 02)



## *References*

### **Calculation of Decontaminant Amount**

$$(10\text{g agent/m}^2) \times (50\text{g decon/g agent}) = 500\text{g decon/m}^2$$

$$(500\text{g decon/m}^2) \times (\text{m}^2/10^4 \text{ cm}^2) = 0.05\text{g decon/cm}^2$$